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Hepatocyte growth factor remains as an inactive single chain after partial hepatectomy or unilateral nephrectomy

Wangxian Tang**, Keiji Miyazawa, Naomi Kitamura*

Institute for Liver Research, Kansai Medical University, Moriguchi, Osaka 570, Japan

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Abstract Hepatocyte growth factor (HGF) is a potent mitogen for hepatocytes and renal tubular epithelial cells. HGF is proteolytically activated in the tissue injured by hepatotoxin or nephrotoxin, suggesting that HGF functions as a crucial growth factor for tissue regeneration following hepatotoxin- or nephrotoxin-induced injury. In this study, we analyzed the molecular form of HGF after partial hepatectomy or after unilateral nephrectomy. The active form of HGF was not detected under our experimental conditions after these operations. Thus, HGF may play little role in liver regeneration after partial hepatectomy and in compensatory renal enlargement after unilateral nephrectomy.

Key words: Hepatocyte growth factor; Proteolytic activation; Partial hepatectomy; Unilateral nephrectomy

1. Introduction

Hepatocyte growth factor (HGF), also known as scatter factor, was initially isolated as a potent mitogen for hepatocytes in primary culture [1]. Mature HGF is produced from a single chain precursor by cleavage at arginine 494 with a serine protease, and consists of a heavy chain and a light chain held together by a disulfide bond [1–3]. The heterodimeric form is required for the mitogenic activity of HGF for hepatocytes in primary culture [4]. HGF also has mitogenic activity upon renal tubular epithelial cells and a variety of cell lines [5–7]. In addition to affecting cellular proliferation, HGF has several other biological effects upon its target cells. It induces motility of epithelial cells, epithelial tubulogenesis and angiogenesis [8–11].

HGF is secreted as an inactive single chain precursor by the producing cells [12], and normally remains in this form associated with the extracellular matrix [13,14]. We found that in response to liver injury induced by hepatotoxins such as carbon tetrachloride and D-galactosamine, the amount of HGF increased and a significant portion of the increased HGF was converted to the active heterodimeric form in the injured liver. Similarly, in response to kidney injury induced by $HgCl_2$, the proteolytic activation of HGF occurred exclusively in the injured kidney. In contrast, the proteolytic activation did not occur in the uninjured tissues [15]. These findings suggested that HGF functions as a crucial growth factor for tissue regeneration following hepatotoxin- or nephrotoxin-induced injury.

Liver regeneration is also induced by partial hepatectomy. Several studies have suggested that HGF plays a crucial role in liver regeneration after partial hepatectomy; the production of HGF is induced in rats after partial hepatectomy [16–18], and the HGF receptor is down-regulated exclusively in the liver after partial hepatectomy [19]. After unilateral nephrectomy, compensatory enlargement of the remaining kidney occurs [20]. Because the level of HGF mRNA is increased and the HGF receptor is down-regulated in the remaining kidney after unilateral nephrectomy, it has been suggested that HGF functions as a mitogenic factor during the compensatory enlargement of the remaining kidney [21]. If HGF is involved in liver regeneration after partial hepatectomy and compensatory renal enlargement after unilateral nephrectomy, it has to be proteolytically activated in the regenerated and enlarged tissues. In this study, we therefore examined the molecular form of HGF in various tissues after partial hepatectomy and unilateral nephrectomy. Under our experimental conditions, we could not detect the active heterodimeric form of HGF, although the amount of HGF increased in various tissues. Thus, HGF may play little role in liver regeneration after partial hepatectomy and in compensatory renal enlargement after unilateral nephrectomy.

2. Materials and methods

2.1. Materials

Reagents were obtained as follows: 6-amidino-2-naphthyl-*p*-guanidino-benzoate dimethansulfonate (nafamostat mesilate) from Torii Co., Ltd; phenylmethylsulfonyl fluoride, and 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS) from Sigma; SP-Sepharose from Pharmacia LKB Biotechnology Inc. The monoclonal antibody against the heavy chain of HGF [12] was provided by the Research Center, Mitsubishi Kasei Corp.

2.2. Preparation of crude HGF from rat tissues

The method of Miyazawa *et al.* [15] was slightly modified. Male rats of Wistar strain (180–220 g) were used in all experiments. Partial hepatectomy was performed by the methods of Higgins and Anderson [22]. After various periods, blood was obtained by cardiac puncture to determine serum alanine aminotransferase [23] and blood urea nitrogen [24]. Then rats were decapitated and the tissues were removed and homogenized in 4 volumes of 50 mM Tris-HCl, pH 8.5, containing 0.15 M NaCl, 10 mM EDTA, 100 μM nafamostat mesilate, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at $25,000 \times g$ for 60 min and the supernatant was centrifuged again at $105,000 \times g$ for 60 min at 4 °C. An aliquot of the supernatant containing 80–150 mg of protein was supplemented with CHAPS to 0.1% and applied to a SP-Sepharose column (0.8×1.6 cm) equilibrated with 20 mM Tris-HCl, pH 8.5, containing 0.15 M NaCl, 0.1% CHAPS and 100 μM nafamostat mesilate. The column was washed with 3 ml of 0.15 M NaCl buffer and 6 ml of 0.4 M NaCl buffer. HGF was eluted with 3 ml of 0.65 M NaCl buffer. The eluate was concentrated by ultrafiltration (Centricon 30, Amicon) after 4-volumes of 40 mM Tris-HCl, pH 6.8, containing 0.1% CHAPS was added to the eluate.

2.3. Immunoblotting

Samples were resolved by SDS-PAGE in the presence of 2-mercaptoethanol on a 7.5% gel according to Laemmli [25]. After SDS-PAGE, proteins were transferred to a polyvinylidene difluoride membrane (Trans-Blot, Bio-Rad). The membranes were incubated with the

*Corresponding author. Fax: (81) (6) 994 6099.

***Present address:* Institute of Liver Disease, Tongji Hospital, Tongji Medical University, Wuhan 430030, People's Republic of China.

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primary monoclonal antibody for 2 h, then with horseradish peroxidase-conjugated antibodies for 2 h. The blots were visualized by means of enhanced chemiluminescence (ECL, Amersham) and exposed to Fuji RX films. The films were scanned and the integrated optical density of bands was determined using a densitometer.

3. Results

We reported a means of quantifying the ratio of single chain to heterodimeric HGF by immunoblotting with an anti-heavy chain monoclonal antibody. We demonstrated by this means that the amount of HGF markedly increased and HGF was proteolytically activated in response to tissue injury [15]. In this study, we used the same means to examine the amount and molecular form of HGF after partial hepatectomy or unilateral nephrectomy.

3.1. After partial hepatectomy

The rats were two thirds hepatectomized, and HGF was extracted from the tissues and analyzed by immunoblotting. The serum alanine aminotransferase activity was increased after the procedure (no surgery, 7.2 ± 1.2; 12 h after partial hepatectomy, 81.4 ± 30.5; 24 h after partial hepatectomy, 56.2 ± 20.2 (mean ± S.D., IU/l), although the level was much lower than that after hepatotoxin treatment. Fig. 1A shows a representative time course of the amount and form of HGF in the remnant liver after partial hepatectomy and in the liver from a sham-operated rat. The amount of HGF increased in the sham-operated rat liver, about twofold over the untreated rat liver. The amount of HGF after partial hepatectomy progressively increased during the first 12 h, and the maximum level was about 14-fold higher than that of the untreated rat liver. The high level was maintained up to at least 24 h. The band corresponding to the heavy chain of HGF was below the detectable level, even when the amount was high. The proteolytic conversion of HGF was obviously observed, when a similar amount of liver HGF from a CCl_4-treated rat was analyzed as a positive control (Fig. 1A, last lane). These results indicated that HGF remained as the inactive single chain form in the remnant liver of the partial hepatectomized rat.

Kono et al. have reported that the level of HGF mRNA increased in the rat kidney after partial hepatectomy, and suggested that the increased HGF is involved in liver regeneration through an endocrine mechanism [26]. We therefore analyzed kidney HGF after partial hepatectomy (Fig. 1B). The amount of HGF increased in the sham-operated rat kidney, about threefold at maximum over the untreated rat kidney. The amount of HGF after partial hepatectomy increased, and reached the maximum level at 12 h, which was about sevenfold higher than that of the untreated rat kidney. Proteolytic conversion into the heterodimeric form was not observed, indicating that the increased HGF remained as the inactive precursor form in the kidney.

3.2. After unilateral nephrectomy

The rats were unilaterally nephrectomized. The level of blood urea nitrogen was not significantly affected by this procedure. Fig. 2A shows a representative time course of the amount and form of HGF in the remaining kidney after unilateral nephrectomy. The amount of HGF increased, and reached the maximum level at 6 h, which was about fivefold higher than that of the untreated rat kidney. The band corresponding to the heavy

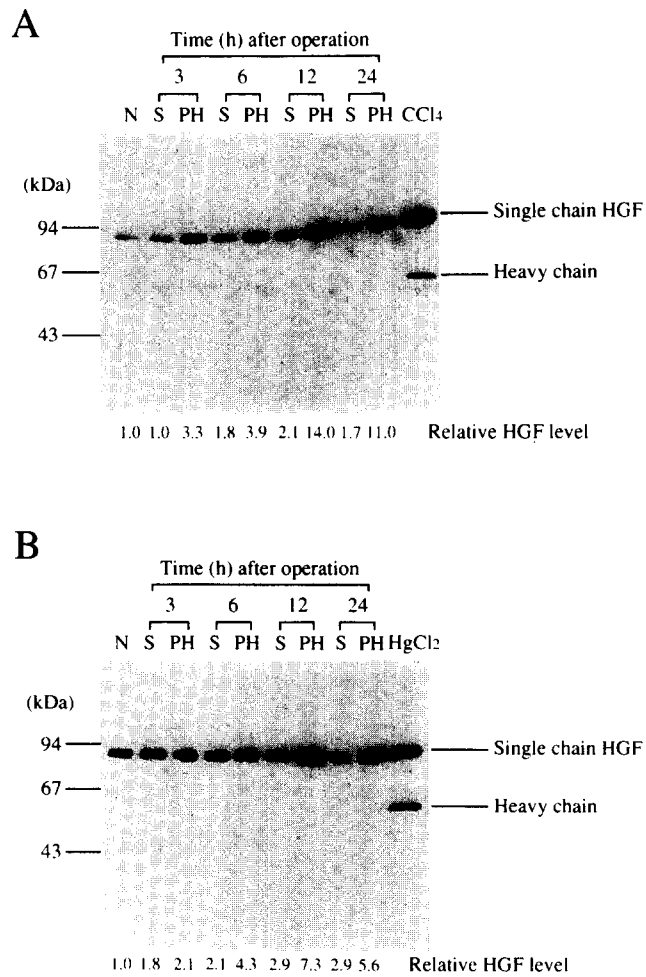

Fig. 1. Immunoblotting of HGF in the remnant liver (A) and in the kidney (B) after partial hepatectomy. Livers and kidneys were removed from normal (N), sham-operated (S) and partially-hepatectomized (PH) rats at various periods after operation, homogenized, then centrifuged. Soluble proteins of the liver (30 mg) and kidney (7.5 mg) were fractionated on SP-Sepharose and immunoblotted. Lane CCl_4 contains 7.5 mg of soluble protein of the liver, which was removed from the rat at 12 h after administration of CCl_4 [15]. Lane $HgCl_2$ contains 5 mg of soluble protein of the kidney, which was removed from the rat at 12 h after administration of $HgCl_2$ [15]. The relative level of HGF is shown below the immunoblots. Molecular mass markers are shown in kilodaltons on the left.

chain of HGF was below the detectable level, even when the amount was high. The proteolytic conversion of HGF was observed, when a similar amount of kidney HGF from a $HgCl_2$-treated rat was analyzed as a positive control (Fig. 2A, last lane). Thus, HGF remained as the inactive single chain form in the remaining kidney after unilateral nephrectomy.

The amount of liver HGF markedly increases after renal injury induced by a nephrotoxin [15]. We analyzed the amount of liver HGF after unilateral nephrectomy (Fig. 2B) and found that it increased, reaching the maximal level at 12 h, which was about tenfold higher than that of the untreated rat liver.

3.3. Spleen and lung HGF after partial hepatectomy or unilateral nephrectomy

The spleen and lung produce HGF in normal rat [27,28]. HGF mRNA levels increase in the rat spleen and lung after

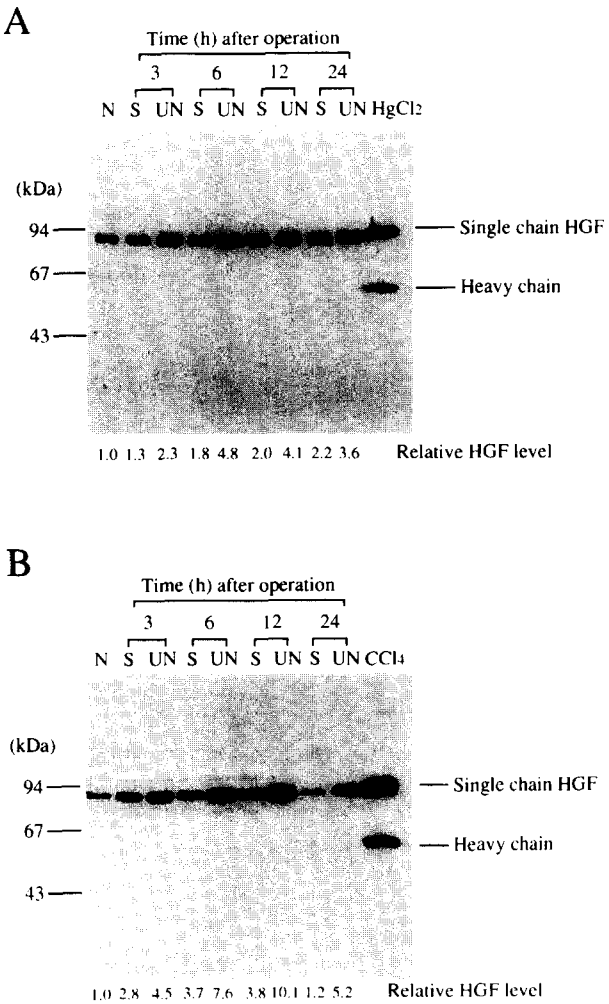

Fig. 2. Immunoblotting of HGF in the remaining kidney (A) and in the liver (B) after unilateral nephrectomy. Kidneys and livers were removed from normal (N), sham-operated (S) and unilateral-nephrectomized (UN) rats. Soluble proteins of the kidney (7.5 mg) and liver (30 mg) were fractionated on SP-Sepharose and immunoblotted. Lanes $HgCl_2$ and CCl_4 contain the sample shown in Fig. 1.

hepatotoxin treatment, partial hepatectomy or unilateral nephrectomy [26,27,29]. HGF protein levels increase in the rat spleen, but not in the lung following hepatotoxin or nephrotoxin treatment [15]. The amount of spleen and lung HGF was analyzed after partial hepatectomy or unilateral nephrectomy (Fig. 3). The amount of HGF markedly increased in the spleen, about tenfold after partial hepatectomy and about ninefold after unilateral nephrectomy more than that of the untreated rat spleen. On the other hand, the amount of HGF was unchanged in the lung after these procedures.

4. Discussion

HGF has a broad spectrum of biological activity upon epithelial cells in vitro. The genes for HGF and its receptor are expressed in many tissues during embryonic preriods and in the adult [27,28,30–34]. However, the physiological functions of HGF in vivo have not yet been fully understood. Previous studies have shown that HGF normally remains as an inactive form in the tissues examined. The inactive form of HGF has to be proteolytically activated to exert its biological activities. Thus, elucidation of the proteolytic activation systems is important for understanding the physiological roles of HGF. One of these systems was found in the liver injured by hepatotoxin [15]. The inactive form of HGF is proteolytically activated in the injured liver and an activity of a serine protease, which is involved in the activation of HGF, is induced exclusively in the injured liver. The activated HGF can physiologically function during liver regeneration, probably as a mitogen for hepatocytes. A similar activation system appears to function in the kidney injured by nephrotoxin, because the active form of HGF is detected in the injured kidney [15]. In this study, to assess the involvement of HGF in liver regeneration after partial hepatectomy and the compensatory enlargement of the remaining kidney after unilateral nephrectomy, we examined the molecular form of HGF after these operations. The results showed that the active form was below the detectable level. Thus, HGF may play little role in the regeneration after tissue resection.

Other studies showed that the HGF receptor was down-regulated in the liver after partial hepatectomy and in the remaining kidney after unilateral nephrectomy [19,20]. However, the present study shows that HGF remains as the inactive form in these tissues. Thus, the down-regulation of the HGF receptor may not result from the HGF binding. Because it is known that the receptor for a growth factor is down-regulated by another growth factor [35,36], the down-regulation of the HGF receptor may be modulated by a growth factor(s) other than HGF, which is involved in liver regeneration or compensatory renal enlargement.

Several growth factors exhibit mitogenic activity for hepatocytes in primary culture. These are epidermal growth factor (EGF) [37], transforming growth factor-α (TGF-α) [38], acidic and basic fibroblast growth factor (aFGF and bFGF) [39,40], keratinocyte growth factor [41] and heparin-binding EGF-like growth factor (HB-EGF) [42]. Among them, EGF, TGF-α and HB-EGF share the same receptor. During liver regeneration after partial hepatectomy in rats, TGF-α and aFGF mRNA increase, and they reach a maximum level at the first peak of DNA synthesis [38,39]. The hepatic level of bFGF increases in the regenerating rat liver during the first 48 h after partial hepatectomy [40]. In the regenerating rat liver on the 3rd day after partial hepatectomy, HB-EGF mRNA levels increase in the non-parenchymal cells [42]. These growth factors may function as mitogens for the further proliferation of hepatocytes beyond the first wave of DNA synthesis. More recently, Mullhaupt et al. reported that within 15 min following partial hepatectomy, EGF mRNA increases over 10-fold, then diminishes to below the basal level prior to the first wave of DNA synthesis in hepatocytes [37]. Thus, EGF may function as an initiator of the first wave of hepatocyte DNA synthesis.

We identified a novel serine protease from human serum, that activates the single chain form of HGF and designated this protease HGF activator [43]. More recently, we found that the HGF-converting activity induced in the injured liver of rat after hepatotoxin treatment is attributed to HGF activator (K. Miyazawa and N. Kitamura, unpublished results). Thus, the induction of the activity of HGF activator is required for the activation of HGF. HGF activator is first produced as an inactive zymogen, which is activated by proteolytic cleavage. Thrombin appears to be responsible for this activation [44]. Thus, cascade reactions may lead to the activation of HGF in

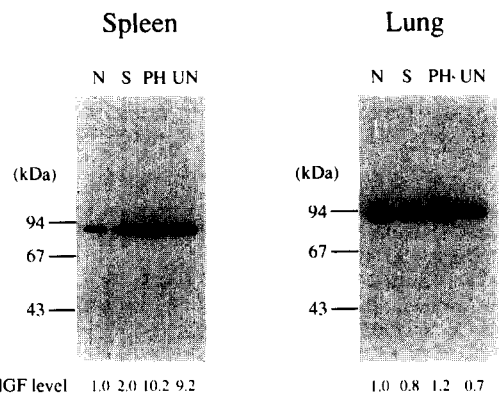

Fig. 3. Immunoblotting of the spleen and lung HGF after partial hepatectomy and unilateral nephrectomy. Spleens and lungs were removed from normal (N), sham-operated (S), partially-hepatectomized (PH) and unilateral-nephrectomized (UN) rats at 12 h after operations. Soluble proteins of the spleen (30 mg) and lung (10 mg) were fractionated on SP-Sepharose and immunoblotted.

the injured liver induced by hepatotoxin. The liver injury leads to the activation of prothrombin, the activated thrombin converts the zymogen of HGF activator to the active form, then the activated HGF activator produces the active form of HGF. Because HGF remains as the inactive form, the activity of HGF activator does not appear to be induced in the liver after partial hepatectomy and in the remaining kidney after unilateral nephrectomy.

The amount of HGF increased in the liver, kidney and spleen but not in the lung after partial hepatectomy and unilateral nephrectomy. Others have shown that HGF mRNA levels increase in the remnant liver, kidney and spleen after partial hepatectomy [16,17,26], and in the remaining kidney, liver and spleen after unilateral nephrectomy [21,26]. Thus, the increase of the amount of HGF protein appears to result from the increase of HGF mRNA in the tissues. Because the liver and kidney are major tissues for storage of HGF protein [15], partial hepatectomy and unilateral nephrectomy lead to a decrease of the storage capacity in the respective tissues. Thus, the increased level of HGF in the untreated tissues may result, in part, from the decrease of HGF storage in the resected tissues. HGF mRNA levels increase in the rat lung after partial hepatectomy and unilateral nephrectomy [29], but the amount of HGF was unchanged in this tissue (in this study). These results indicated that the lung has no capacity of HGF storage above the normal level.

Even though HGF was not proteolytically activated after partial hepatectomy and unilateral nephrectomy, HGF mRNA was induced in a variety of tissues. Tissue resection leads to disturbances of normal homeostasis and subsequently various responses, such as inflammatory response, occur to maintain homeostasis. Because inflammatory cytokines, such as interleukin-1 and tumor necrosis factor-α, induce HGF gene expression in cultured cells [45], these factors may be responsible for the induction after tissue resection. However, when subcutaneous acute inflammation was induced by turpentine, the amount of HGF did not increase in the liver and kidney [15]. Thus, the induction of HGF mRNA does not appear to result only from inflammatory response.

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References

[1] Gohda, E., Tsubouchi, H., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Miyazaki, H., Hashimoto, S. and Daikuhara, Y. (1988) J. Clin. Invest. 81, 414–419.
[2] Miyazawa, K., Tsubouchi, H., Naka, D., Takahashi, K., Okigaki, M., Arakaki, N., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Gohda, E., Daikuhara, Y. and Kitamura, N. (1989) Biochem. Biophys. Res. Commun. 163, 967–973.
[3] Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K. and Shimizu, S. (1989) Nature 342, 440–443.
[4] Naka, D., Ishii, T., Yoshiyama, Y., Miyazawa, K., Hara, H., Hishida, T. and Kitamura, N. (1992) J. Biol. Chem. 267, 20114–20119.
[5] Igawa, T., Kanda, S., Kanetake, H., Saitoh, Y., Ichihara, A., Tomita, Y. and Nakamura, T. (1991) Biochem. Biophys. Res. Commun. 174, 831–838.
[6] Rubin, J.S., Chan, A.M.-L., Bottaro, D.P., Burgess, W.H., Taylor, W.G., Cech, A.C., Hirschfield, D.W., Wong, J., Miki, T., Finch, P.W. and Aaronson, S.A. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 415–419.
[7] Kan, M., Zhang, G., Zarnegar, R., Michalopoulos, G., Myoken, Y., McKeehan, W.L. and Stevens, J.I. (1991) Biochem. Biophys. Res. Commun. 174, 331–337.
[8] Weidner, K.M., Arakaki, N., Hartmann, G., Vandekerckhove, J., Weingart, S., Rieder, H., Fonatsch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y. and Birchmeier, W. (1991) Proc. Natl. Acad. Sci. USA 88, 7001–7005.
[9] Montesano, R., Matsumoto, K., Nakamura, T. and Orci, L. (1991) Cell, 67, 901–908.
[10] Bussolino, F., Di Renzo, M.F., Ziche, M., Bocchietto, E., Olivero, M., Naldini, L., Gaudino, G., Tamagnone, L., Coffer, A., and Comoglio, P.M. (1992) J. Cell Biol. 119, 629–641.
[11] Grant, D.S., Kleinman, H.K., Goldberg, I.D., Bhargava, M.M., Nickoloff, B.J., Kinsella, J.L., Polverini, P. and Rosen, E.M. (1993) Proc. Natl. Acad. Sci. USA 90, 1937–1941.
[12] Miyazawa, K., Kitamura, A., Naka, D. and Kitamura, N. (1991) Eur. J. Biochem. 197, 15–22.
[13] Masumoto, A. and Yamamoto, N. (1991) Biochem. Biophys. Res. Commun. 174, 90–95.
[14] Naldini, L., Tamagnone, L., Vigna, E., Sachs, M., Hartmann, G., Birchmeier, W., Daikuhara, Y., Tsubouchi, H., Blasi, F. and Comoglio, P.M. (1992) EMBO J. 11, 4825–4833.
[15] Miyazawa, K., Shimomura, T., Naka, D. and Kitamura, N. (1994) J. Biol. Chem. 269, 8966–8970.
[16] Selden, C., Jones, M., Wade, D. and Hodgson, H. (1990) FEBS Lett. 270, 81–84.
[17] Zarnegar, R., DeFrances, M.C., Kost, D.P., Lindroos, P. and Michalopoulos, G.K. (1991) Biochem. Biophys. Res. Commun. 177, 559–565.
[18] Kinoshita, T., Hirao, S., Matsumoto, K. and Nakamura, T. (1991) Biochem. Biophys. Res. Commun. 177, 330–335.
[19] Tajima, H., Higuchi, O., Mizuno, K. and Nakamura, T. (1992) J. Biochem. 111, 401–406.
[20] Johnson, H.A. and Roman, J.M.V. (1966) Am. J. Pathol. 49, 1–13.
[21] Nagaike, M., Hirao, S., Tajima, H., Noji, S., Taniguchi, S., Matsumoto, K. and Nakamura, T. (1991) J. Biol. Chem. 266, 22781–22784.
[22] Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186–202
[23] Wróblewski, F. and La Due, J.S. (1956) Proc. Soc. Exper. Biol. Med. 91, 569–571.
[24] Coulombe, J.J. and Favreau, L. (1963) Clin. Chem. 9, 102–108.
[25] Laemmli, U.K. (1970) Nature 227, 680–685.
[26] Kono, S., Nagaike, M., Matsumoto, K. and Nakamura, T. (1992) Biochem. Biophys. Res. Commun. 186, 991–998.
[27] Okajima, A., Miyazawa, K. and Kitamura, N. (1990) Eur. J. Biochem. 193, 375–381.
[28] Tashiro, K., Hagiya, M., Nishizawa, T., Seki, T., Shimonishi, M.,

Shimizu, S. and Nakamura, T. (1990) Proc. Natl. Acad. Sci. USA 87, 3200–3204.
[29] Yanagita, K., Nagaike, M., Ishibashi, H., Niho, Y., Matsumoto, K. and Nakamura, T. (1992) Biochem. Biophys. Res. Commun. 182, 802–809.
[30] Defrances, M.C., Wolf, H.K., Michalopoulos, G.K. and Zarnegar, R. (1992) Development 116, 387–395 .
[31] Chan, A.M.-L., King, H.W.S., Deakin, E.A., Tempest, P.R., Hilkens, J., Kroezen, V., Edwards, D.R., Wills, A.J., Brookes, P. and Cooper, C.S. (1988) Oncogene 2, 593–599.
[32] Iyer, A., Kmiecik, T.E., Park, M., Daar, I., Blair, D., Dunn, K.J., Sutrave, P., Ihle, J.N., Bodescot, M. and Vande Woude, G.F. (1990) Cell Growth & Differ. 1, 87–95.
[33] Di Renzo, M.F., Narsimhan, R.P., Olivero, M., Bretti, S., Giordano, S., Medico, E., Gaglia, P., Zara, P. and Comoglio, P.M. (1991) Oncogene 6, 1997–2003.
[34] Sonnenberg, E., Meyer, D., Weidner, K.M. and Birchmeier, C. (1993) J. Cell Biol 123, 223–235.
[35] Wharton, W., Leof, E., Pledger, W.J. and O'Keefe, E.J. (1982) Proc. Natl. Acad. Sci. USA 79, 5567–5571.
[36] Olashaw, N.E., O'Keefe, E.J. and Pledger, W.J. (1986) Proc. Natl. Acad. Sci. USA 83, 3834–3838.
[37] Mullhaupt, B., Feren, A., Fodor, E. and Jones, A. (1994) J. Biol. Chem. 269, 19667–19670.
[38] Mead, J.E. and Fausto, N. (1989) Proc. Natl. Acad. Sci. USA 86, 1558–1562.
[39] Kan, M., Huang, J., Mansson, P-E., Yasumitsu, H., Carr, B. and McKeehan, W.L. (1989) Proc. Natl. Acad. Sci. USA 86, 7432–7436.
[40] Presta, M., Statuto, M., Rusnati, M., Dell'Era, P. and Ragnotti, G. (1989) Biochem. Biophys. Res. Commun. 164, 1182–1189.
[41] Itoh, T., Suzuki, M. and Mitsui, Y. (1993) Biochem. Biophys. Res. Commun. 192, 1011–1015.
[42] Ito, N., Kawata, S., Tamura, S., Kiso, S., Tsushima, H., Damm, D., Abraham, J.A., Higashiyama, S., Taniguchi, N. and Matsuzawa, Y. (1994) Biochem. Biophys. Res. Commun. 198, 25–31.
[43] Miyazawa, K., Shimomura, T., Kitamura, A., Kondo, J., Morimoto, Y. and Kitamura N. (1993) J. Biol. Chem. 268, 10024–10028.
[44] Shimomura, T., Kondo, J., Ochiai, M., Naka, D., Miyazawa, K., Morimoto, Y. and Kitamura, N. (1993) J. Biol. Chem. 268, 22927–22932.
[45] Tamura, M., Arakaki, N., Tsubouchi, H., Takada, H. and Daikuhara, Y. (1993) J. Biol. Chem. 268, 8140–8145.